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released from from nuclear fuel reprocessing plants***

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MICROBIAL TRANSFORMATIONS OF RADIONUCLIDES RELEASED FROM NUCLEAR FUEL REPROCESSING PLANTS

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ABSTRACT

Microorganisms can affect the stability and mobility of the actinides U, Pu, Cm, Am, Np, and the fission products Tc, I, Cs, Sr, released from nuclear fuel reprocessing plants. Under appropriate conditions, microorganisms can alter the chemical speciation, solubility and sorption properties and thus could increase or decrease the concentrations of radionuclides in solution and the bioavailability. Dissolution or immobilization of radionuclides is brought about by direct enzymatic action or indirect non-enzymatic action of microorganisms. Although the physical, chemical, and geochemical processes affecting dissolution, precipitation, and mobilization of radionuclides have been investigated, we have only limited information on the effects of microbial processes. The mechanisms of microbial transformations of the major and minor actinides and the fission products under aerobic and anaerobic conditions in the presence of electron donors and acceptors are reviewed.

Key words: Actinides, fission products, microorganisms, biotransformation, mobilization, immobilization.

1. INTRODUCTION

Radionuclides released from nuclear fuel processing plants of concern are the major (U and Pu) and minor (Am, Np, Cm) actinides, and the fission products (I, Cs, Sr, and Tc). The radionuclides released may be in various forms and may eventually exist in the environment as elemental, oxide, coprecipitates, ionic, inorganic-, and organic-complexes. Microbial activity could also affect the chemical nature of the radionuclides by altering the speciation, solubility and sorption properties and thus could increase or decrease the concentrations of radionuclides in solution and their bioavailability. Under appropriate conditions, dissolution or immobilization of radionuclides is brought about by direct enzymatic or indirect non-enzymatic actions of microorganisms. These include (i) oxidation-reduction reactions, (ii) changes in pH and Eh, (iii) chelation or production of specific sequestering agents, (iv) bioaccumulation by biomass, (v) biocolloid formation, (vi) bioprecipitation, (vi) biotransformation of radionuclide-organic and -inorganic complexes, and (vii) production of volatile compounds by biomethylation (Francis 1984; 1990). Among the radionuclides, biotransformation of uranium has been extensively studied, whereas we have only limited understanding of the microbial transformations of other radionuclides generated from nuclear reprocessing plants.

Dissolution of metals by heterotrophic microorganisms is due to production of organic acid metabolites, as well as lowering of the pH of the medium from the metabolism of organic compounds. In many cases, a combined effect is important; for example, when organisms secrete organic acids they may have the dual effect of increasing metal dissolution by lowering pH and complexation. Heterotrophic bacteria and fungi are not only known to solubilize various minerals including silicates (quartz, feldspar, mica) but also release metals associated with them,

including Cu and Ni from copper-nickel concentrates, Cu from low-grade copper ore, uranium from granites, and potassium from leucite. Microbially produced dicarboxylic acids, oxalic, isocitric, citric, succinic, ketogluconic acid, polyhydroxy acids, and phenolic compounds such as protocatechuic acid, and salicylic acid are effective chelating agents of metals and are known to accelerate their movement in soils (Berthelin and Munier-Lamy, 1983; Bloomfield and Pruden, 1975). Iron-sequestering agents such as siderophores elaborated by microorganisms apparently play an important role in the complexation of metals and increase their solubility. Very little is known though of similar effects on Sr and Cs dissolution in soils.

Metals associated with or coprecipitated with iron and manganese oxides and hydroxides can be remobilized due to reduction of host metal Fe^{3+} and Mn^{4+} (hydro)oxides either chemically or enzymatically (Stone and Morgan, 1987; Francis and Dodge, 1990) under anaerobic conditions. Microbially mediated metal reductions play an important role in the biogeochemical cycles of metals, and can also lead to the remobilization of sorbed metals such as Cd, Cr, Ni, Pb, and Zn (Francis and Dodge 1990). Although Sr and Cs are not redox reactive they can be remobilized and made bioavailable through reductive dissolution of iron hydroxide hosts. In this paper, biotransformation of various chemical species of the radionuclides in the environment is reviewed.

2. BIOTRANSFORMATION OF URANIUM

Uranium exists in U(III), U(IV), U(V), and U(VI) oxidation states, of which U(IV) and U(VI) are the predominant ones in the environment. Both aerobic and anaerobic microorganisms are involved in the mobilization and immobilization of various chemical forms of uranium. Dissolution of uranium by autotrophic sulfur- and iron-oxidizing bacteria due to production of sulfuric acid and by heterotrophic bacteria and by fungi due to production of organic acids and chelating agents has been well documented in the literature (Francis 1990). The direct implication of microorganisms in the biotransformation of uranium is of considerable interest because of its potential application in bioremediation of contaminated sites, in pre-treating radioactive wastes, and in processes critical to the performance of nuclear waste repositories.

2.1. Mobilization of Uranium. The iron and sulfur oxidizing bacteria play a significant role in the solubilization of uranium from ores and in mill tailings. The biogeochemistry of uranium recovery from ores and bacterial leaching has been extensively studied. The role of autotrophic bacteria *Thiobacillus ferrooxidans* in the extraction of uranium from ore is primarily indirect action due to generation of the oxidizing agent ferric sulfate and the solvent sulfuric acid.

2.2. Immobilization of Uranium. The immobilization of uranium is brought about by bioaccumulation, bioreduction and bioprecipitation reactions. Uranium is reduced by a wide variety of facultative and strict anaerobic bacteria under anaerobic conditions in the presence of suitable electron donor. Consequently, the potential exists for the use of anaerobic bacteria to concentrate, contain and stabilize uranium in contaminated groundwaters and in waste with concurrent reduction in waste volume. However, the long-term stability of bacterially reduced uranium in the natural environment is not known.

2.2.1. Biosorption and Bioaccumulation of Uranium. Biosorption and bioaccumulation of uranium has been observed in a wide range of microorganism (Sakaguchi, 1996; Strandberg et al 1981). It has been and perhaps it still is one of the intensely investigated areas of research because of the potential use of biomass to remove uranium from waste streams. Uranium forms complexes with the carboxylate, phosphate, amino, and hydroxyl functional

groups present on the cell surface; and intracellularly, by binding to anionic sites or precipitating as dense deposits. Nuclear magnetic resonance spectroscopy (NMR), time resolved laser fluorescence spectroscopy (TRLFS), and extended X-ray fluorescence spectroscopy (EXAFS) have been used to determine the functional groups involved in the complexation of U with bacteria.

Extracellular and intracellular association of U with bacteria was observed but the extent of its accumulation differs greatly with the species of bacteria. Extracellular association of uranium with bacterial cell surfaces is primarily due to physical- and chemical- interactions involving adsorption, ion exchange, and complexation and does not depend on metabolism. Bacterial cell walls, exopolymers, proteins, and lipids contain functional groups, which are able to bind uranium. In *Halomonas* sp. U accumulated as electron-dense intracellular granules and was also bound to the cell surface (Figure 1). EXAFS analysis of the association of U with halophilic and non-halophilic bacterial cells showed that it was associated predominantly with phosphate as uranyl hydrogen phosphate and additional forms of phosphate such as hydroxophosphato or polyphosphate complexes as well as other ligands such as carboxyl species (Francis et al., 2004). These results demonstrate that phosphate, including the polyphosphates, bind significant amounts of uranium in bacteria.

Intracellular accumulation involves transporting the metal across the cell membrane, which depends on the cell's metabolism. The intracellular transport of the U into the cell involves an as-yet unidentified transport system. Uranium transport across bacterial cell membranes may be mediated by the mechanism used to convey metabolically essential ions; however, additional studies are needed to clarify the exact mechanism involved.

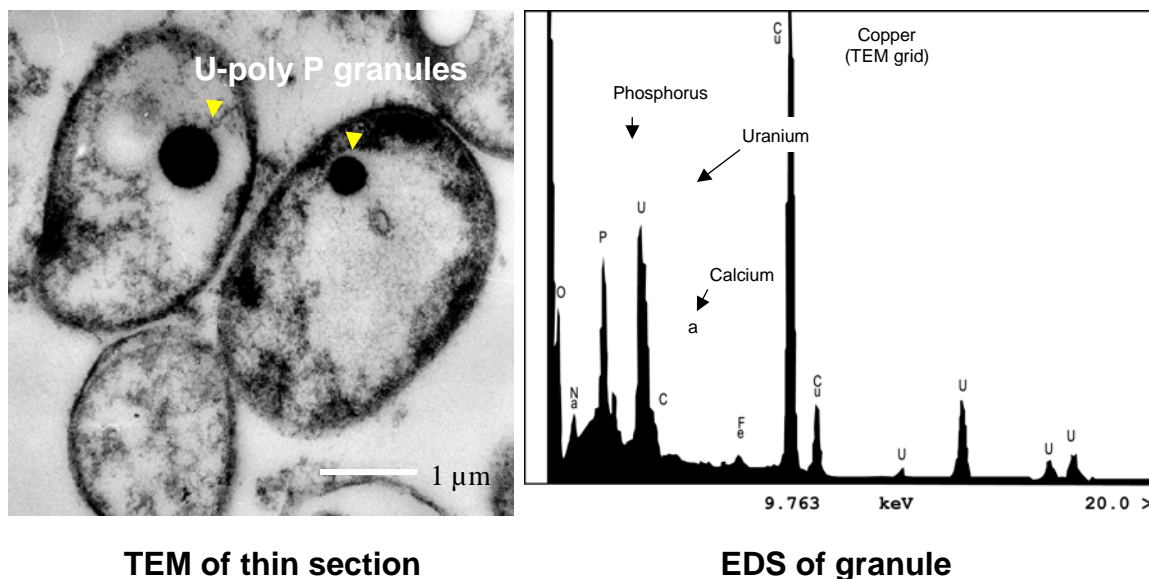


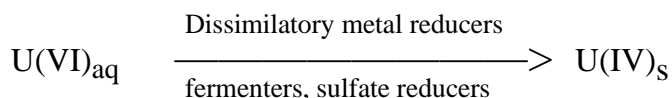
Figure 1. Intra- and extra- cellular accumulation of Uranium by *Halomonas* sp. After exposure to U, EDS shows U and P as the major constituents of the intracellular granules (Francis et al., 2004).

Polyphosphates are widely distributed throughout the bacterial cell. Numerous and varied biological functions are performed by polyphosphate including phosphate storage in the cell, a reservoir of energy for cellular functions, a chelator of metals (e.g., Mn^{2+} and Ca^{2+}), a pH buffer, a capsule for bacteria, and in physiological adjustments to growth, development, stress, and

deprivation. In particular, the polyphosphates play a vital role in the dynamics of metabolic adjustments of cells to stationary phase and their survival in response to a variety of nutritional limitations and environmental stresses. The amount of polyphosphate that is stored by cells varies between bacterial species, and is determined in part by the rate at which it can be degraded for example, in response to the presence of metals, and the amount of inorganic phosphate secreted into the medium. In as much as all of uranium exposure studies reported were conducted with cells in the stationary phase, the cells are responding to heavy metal stress by releasing phosphate from the mineralization of cellular polyphosphate. In some experiments reported in the literature the cells were in fact incubated with uranium from several hours to days. Under these conditions, the cells undergo lysis and release inorganic phosphate (H_2PO_4^-) with the precipitation of uranium as uranyl phosphate [$\text{UO}_2(\text{H}_2\text{PO}_4)_2$]. Furthermore, in the presence of uranium a decrease in the free phosphate concentration due to its precipitation with uranium would accelerate the rate and extent of polyphosphate degradation. These results show that an increase in the concentration of inorganic phosphate released from mineralization of cellular phosphates during starvation or under stress bind significant amounts of uranium.

Uranium associated with the bacteria is not very stable, as it was removed completely by Na_2HCO_3 from *Halomonas* sp, from an *Arthrobacter* sp by 0.1 sodium hydrogen carbonate, 0.1M EDTA, and Na_2CO_3 , and from *Bacillus* strains by EDTA. Although bacteria possess a variety of functional groups, studies suggest that cellular phosphate is the predominant functional group complexes with U. Inorganic phosphate generated inside cells during starvation or under stress bind U and other cations.

2.1.2. Bioreduction of uranium. A wide variety of facultative and strict anaerobic bacteria reduced U(VI) added as uranyl-nitrate or uranyl carbonate to U(IV) under anaerobic conditions (Francis, 1994; Francis et al., 1994). These include axenic cultures of iron-reducing, fermentative, and sulfate-reducing bacteria. Mixed cultures of bacteria in uranium contaminated ground waters and in wastes also reduced uranium.



Clostridia are strict anaerobic spore-forming fermentative bacteria ubiquitous in soils, sediments, and wastes catalyze the reduction of metals from higher to lower oxidation state. Reduction of soluble U(VI) to insoluble U(IV) by the anaerobic bacterium, *Clostridium* sp. in culture medium was confirmed by X-ray absorption near edge spectroscopy (XANES) and X-ray photoelectron spectroscopy (XPS).

The presence of organic ligands affected the extent of precipitation of reduced uranium under anaerobic conditions. For example, *Clostridium* sp. (ATCC 53464) which ferments glucose but not citrate reduced U(VI)-citrate or U(VI)-phthalate only when supplied with glucose (Francis et al. 2002; Vazquez and Francis, manuscript in preparation). Also the sulfate-reducing bacteria *Desulfovibrio desulfuricans* and the facultative iron-reducing bacteria *Shewanella halotolerans* reduced U(VI) complexed with oxalate or citrate to U(IV) under anaerobic conditions with little precipitation of uranium (Ganesh et al., 1997). These results show that complexed uranium is readily accessible for the microorganisms as an electron acceptor, despite

the inability of the bacteria to metabolize the organic ligand complexed to the actinide and that reduced uranium complexed with the organic ligand remained in solution as soluble species.

3. BIOTRANSFORMATION OF PLUTONIUM

Plutonium can exist in several oxidation states (III, IV, V, VI, VII) and the solution chemistry is very complex. Plutonium (IV) is the predominant form found in contaminated soils. Plutonium can simultaneously coexist as Pu(IV), Pu(V), and Pu(VI) in oxic environments.. Plutonium has a high ionic charge, and tends to undergo hydrolysis, leading to the formation of polymers in systems with pH>2. Soil pH, its organic-matter content, redox conditions, and mineralogy affect the chemical speciation of Pu. Chemical characterization of Pu at contaminated sites shows that its environmental form varies according to site, and depends on the waste stream. For example, at Rocky Flats, CO, the predominant form appears to be PuO₂(s); at the NTS, Pu was found to be associated with mineral colloids (Clark et al 2006; Kersting et al. 1999). Pu generally is considered to be relatively immobile; however, the transport of Pu, albeit at very low concentrations, was observed at several DOE sites (i.e., Rocky Flats, LANL, and NTS).

Microorganisms may directly or indirectly affect the oxidation and reduction of Pu (Figure 2). For example, a slight increase in microbial activity (respiration) can alter the oxidation state of Pu(VI) to Pu(IV) because of the very small differences in the reduction potential between Pu(VI), Pu(V), and Pu(IV). The direct enzymatic reduction of Pu(VI) and Pu(V) to Pu(IV) by bacterial cell suspension of *Shewanella putrefaciens*, *S. oneidensis*, and *Geobacter metallireducens* have been reported (Neu et al 2005). Bioreduction of Pu(IV) to Pu(III) by *Bacillus* sp has been inferred (Rusin et al 1994). Recently, reductive dissolution of Pu(IV) to Pu(III) by the strict anaerobic bacterium *Clostridium* sp was shown by Francis et al, (*manuscript in preparation*) and the Pu(III) oxidation state was confirmed by x-ray absorption near edge spectroscopy (XANES). The potential exists for the microbial oxidative dissolution of the more stable and environmentally predominant form of Pu(IV) to the more soluble and bioavailable form of Pu(V) and Pu(VI). The microbes may indirectly affect the oxidation state and solubility of Pu by changing the Eh and pH of the medium, as well as by producing sequestering agents (Brainard et al. 1992; Francis 2001; John et al 2001, Neu et al 2002; 2005).

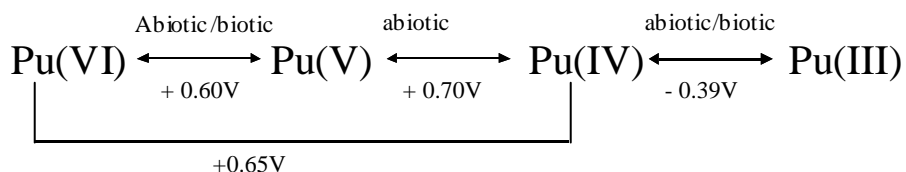


Figure 2. Plutonium oxidation states and redox potentials at pH 8 (Choppin, 1999).

Sorption studies of Pu(VI) with bacterial biomass have shown that the interaction with bacteria can cause changes in the oxidation state (Panak and Nitsche, 2001; Ohnuki et al., *manuscript submitted*) and most of the Pu(VI) was reduced to Pu(IV) and bound to the phosphate groups on the cell surface (Panak et al 2002). Knowledge of plutonium complexes formed with bacterial cells and metabolic products are important in assessing the migration behavior and transport of Pu from contaminated environments to humans.

4. BIOTRANSFORMATION OF NEPTUNIUM

Neptunium is one of the minor actinides generated from the reprocessing of nuclear fuels. It exists in solution primarily as the Np^{5+} species that forms stable carbonate complexes. In general, the pentavalent species of all actinides are unstable, except for Np(V) which is the common form in some natural waters. The chemical characteristics of pentavalent actinides, for example NpO^{2+} , are similar to those of simple monovalent cations: i.e., low ligand-complexing abilities with a high environmental mobility. Neptunyl species (NpO_2^+) which is mobile, non-sorptive can be biologically reduced to insoluble Np^{4+} under anaerobic conditions (Lloyd et al., 2000; Rittmann et al., 2002; Nagaoka, 2005; Neu et al., 2005). Complexation of Np(IV) by fermentation intermediate products prevented its precipitation (Rittmann et al 2002). Although Np^{4+} is easily oxidized in solution, it is stabilized in the presence of complexing ligands. For example, *Shewanella putrefaciens* reduced Np^{5+} to Np^{4+} that then was precipitated from solution as Np^{4+} phosphate (Lloyd et al., 2000). Reduction of Np(V) to Np(IV) by cell suspension of *S. putrefaciens* MR-1 (Neu et al 2005) and by the sulphate-reducing bacteria *Desulfovibrio desulfuricans* (Nagaoka, 2005) have been reported. Neptunyl (NpO_2^+), which is generally thought to be non-sorptive showed significant sorption by *Pseudomonas fluorescens* cells (Songakasiri, et al. 2002). XANES analysis of Np associated with the cells showed no reduction Np(V) . This is in contrast to previous studies which showed negligible uptake of Np by *P. aeruginosa*, *Streptomyces viridochromogenes*, *Scenedesmus obliquus*, and *Micrococcus luteus* (Strandberg and Arnold, 1988).

5. BIOTRANSFORMATION OF AMERICIUM

Americium can exist in multiple oxidation states Am (III, IV, V, VI, VII); but under environmental conditions, Am(III) is the most stable and predominant form. The principle oxidation state of Am is 3+, with the Am(OH)_3 species being extremely surface-reactive. The presence of soluble carbonate complexes, specifically AmCO_3^+ , $\text{Am(CO}_3)_2^-$, and $\text{Am(CO}_3)_3^-$ is well established. Microbial activity may convert Am(OH)^{2+} species to Am(III)-carbonate complexes due to carbon dioxide production; likewise, biodegradation of Am(III)-organic complexes may precipitate Am as the hydroxide species. Sorption of Am to biomass has been reported. Bacteria isolated from sediments and grown with ^{241}Am in minimal medium produced exometabolites, which formed soluble complexes with Am (Geisy et al. 1977). Thus, the potential exists for dissolution of Am in wastes by microorganisms, thereby increasing its bioavailability and mobility.

6. BIOTRANSFORMATION OF CURIUM

Biosorption of Cm(III) on the surface of microorganisms *Chlorella vulgaris*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Halomonas* sp, *Halobacterium salinarum* and *Halobacterium halobium* (Ozaki et al 2004) and sulfate reducing bacteria *Desulfovibrio* (Moll et al 2004) have been reported. No incorporation of Cm(III) into the bacterial cells was found and that the presence of organic compounds in the exudates of microorganisms affected the biosorption and coordination of Cm with the bacterial cells.

7. BIOTRANSFORMATION OF TECHNETIUM

Technetium is produced in large quantities by the fission of ^{235}U during nuclear power generation and defense related activities including nuclear testing and reactor operations (Yoshihara, 1996). At present there are 19 known isotopes with mass numbers from 92 to 107 with half-lives ranging from a few seconds to several hundred thousand years (2.1×10^5 years). Tc can exist in oxidation states 0, +3, +4, +5, +6, and +7; however, the predominant chemistry concerns only the stable heptavalent pertechnetate ion (TcO_4^-) and the quadrivalent Tc(IV) ion. Its chemical behavior resembles that of rhenium. Technetium is readily reduced and oxidized. Uncomplexed Tc(IV,III) undergoes hydrolysis and precipitates from solution. Tc(V) and Tc(VI) undergoes disproportionation reactions to form Tc(IV) and Tc(VII) . The very soluble pertechnetate ion is precipitated from dilute hydrochloric acid (up to 5 M) by hydrogen sulfide and the insoluble reduced form is oxidized to the soluble pertechnetate anion by hydrogen peroxide in alkaline solution.

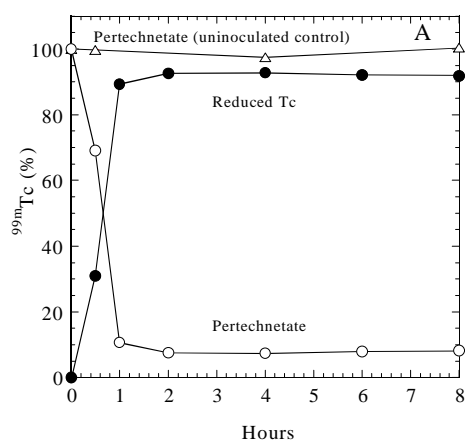
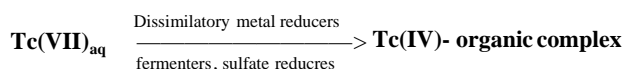
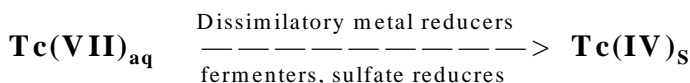


Figure 3. Reduction of pertechnetate by *Clostridium sphenoides* (Francis et al., 2002).

sphenoides capable of citrate and glucose metabolism and *Clostridium* sp. capable of fermenting glucose but not citric acid reduced Tc(VII) to Tc(IV) (Figure 3). The reduced Tc was predominantly associated with the cell biomass. It also was present in solution complexed with bacterial metabolic products ($\text{MW} > 5000$). Addition of diethylenetriamine pentaacetic acid (DTPA) to *Clostridium* sp. resulted in the formation of a soluble Tc(IV) -DTPA complex (Francis et al., 2002). Technetium absorption by soil has been attributed to soil microbial activity (Landa et al., 1976). It has been found that 98% of technetium is absorbed within 2 to 5 weeks by eight of the eleven soils and sterilization of the soil eliminated this absorption. Peretrukhin et al. (1996) reported technetium sorption by bottom sediments of a lake in Russia due to microbial sulfate reduction. Biogenic hydrogen sulfide converts the initial readily soluble sodium pertechnetate to poorly soluble technetium (VII) and technetium (IV) sulfides.

Speciation of microbially reduced Tc. The predominant reduced Tc species identified include TcO_2 , Tc(OH) , TcS_2 depending on the type of microorganism involved. For example, the sulfate reducers generate H_2S , which results in the reduction and precipitation of Tc as TcS , whereas with non-sulfate reducers it is present as Tc oxide and hydroxide species. However there

Microorganisms affect the dissolution or precipitation of Tc by oxidation-reduction reactions and by complexation with organic by-products and macromolecules. Chemolithotrophic, haloalkaliphilic, aerobic, facultative, and anaerobic (fermentative and sulfate-reducing) bacteria reduced pertechnetate ion to an insoluble form (Lyalikova and Khizhnyak, 1996; Pignolet et al., 1989; Tagami and Uchida, 1996; Wildung et al., 2000; Lloyd and Macaskie, 1996; Henrot, 1989; Francis et al. 2002; Khijniak et al., 2003). For example, *Clostridium*



is a significant amount of Tc bound to the cells and also with macromolecules, which could affect the long-term stability and mobility of reduced Tc complexed with organic ligands. We found that a substantial amount of reduced Ts is associated with bacterial cells and with bacterial macromolecule most probably as an organic complex. The potential exist for colloidal transport of the reduced Tc-organic complexes. The nature and stability of the Tc-organic complexes is not known. Tc may be present as insoluble or soluble form or as colloids, depending on the type and extent of bacterial activity in subsurface environments and therefore, the potential exists for the transport of reduced Tc in these forms

8. BIOTRANSFORMATION OF CESIUM AND STRONTIUM

Bioaccumulation of Sr and Cs (structural analogues for Ca and K, respectively), has been reported for several microorganisms (Faison, et al. 1990; Tomioka, et al. 1992). Consequently, there is considerable interest in using microorganisms to remove radioactive Sr and Cs from waste streams and contaminated sites. Mixed cultures of bacteria, isolated from low-level radioactive waste leachates preferentially accumulated ^{85}Sr in mineral salts medium containing a mixture of radionuclides (Francis 1985). The bacteria accumulated $^{85}\text{Sr} > ^{60}\text{Co} > ^{137}\text{Cs}$. Similarly, ^{137}Cs and ^{226}Ra are concentrated less than uranium by microbial cultures (Strandberg et al., 1981). Sr-binding activity in *Micrococcus luteus* is localized on the cell envelope and is sensitive to pretreatment. Bound Sr can be displaced by chelating agents, divalent cations or H^+ (other monovalent cations are less effective at displacing Sr). Sr binding in *M. luteus* is reversible, though both ion exchange, mediated by acidic cell surface components and intracellular uptake may be involved (Faison, et al., 1990).

Cesium-accumulating bacteria isolated from soil (Tomioka, et al., 1992) display the rod-coccus growth cycle and contain mesodiaminopimelic acid, mycolic acids, and tuberculostearic acids. Cs uptake is optimal at pH 8.5 and cannot be modeled as simple sorption. Potassium and rubidium inhibit Cs accumulation suggesting that Cs is taken up through the potassium transport system (Tomioka, et. al., 1994). Nevertheless, the nature of Cs association with the cells (extra or intra cellular) remains unclear, as does the long-term fate of bioassociated Cs.

In soils ^{90}Sr and ^{137}Cs is likely to be present as an exchange form or bound to soil organic matter, iron (hydr)oxides, or insoluble carbonate or phosphate. Microorganisms can affect the association of above mentioned forms of Sr and Cs in soils in the following way: (i) dissolution of carbonate and phosphate phases, clays, and other minerals due to production of organic acids and sequestering agents; (ii) reductive dissolution of iron and the release of Sr and Cs coprecipitated or associated with the iron oxides, (iii) biodegradation of the organic carbon associated Sr and Cs fractions; (iv) immobilization due to precipitation reactions i.e., formation of strontium carbonate, microbial formation of strontium calcite phase and by biomass/exopolymers. The nature of the association of Sr and Cs in soil and the mechanisms of remobilization due to aerobic and anaerobic microbial activity is not fully understood.

9. TRANSFORMATION OF IODINE

Iodine-129 (half life 1.57×10^7 y) is one of the persistent radionuclides released into the environment from nuclear fuel processing activities. The predominant aqueous chemical forms of iodine (I_2), iodide (I^-) and iodate (IO_3^-) are highly soluble and mobile in the environment. Microorganisms are known to affect the chemical behavior of iodine through processes such as volatilization (CH_3I), oxidation of I^- to I_2 , reduction of IO_3^- to I^- , and bioaccumulation by

bacterial cells both intracellularly and extracellularly. Microbial volatilization of organic iodine was observed in soil slurries and seawater samples by aerobic bacteria through methylation of iodide (I^-) to form methyl iodide (CH_3I). The volatilization of iodide was also found in iodide-rich natural brine water. In addition to the organic iodine compounds, a significant amount of molecular iodine (I_2) was produced. Axenic cultures of bacteria are known to produce diiodomethane (CH_2I_2) and chloriodomethane (CH_2ClI). Iodide-oxidizing bacteria, which oxidize I^- to I_2 were isolated from seawater and natural brine water. Sulfate-reducing bacteria *Desulfovibrio desulfuricans* and metal reducing bacteria *Shewanella putrefaciens* have been shown to reduce iodate to iodide. Iodate (IO_3^-) is electrochemically or biologically reduced to I^- prior to uptake by rice plants. Changes in iodine redox states could have important effects on the mobility of iodine in natural systems. Conditions that are known to influence microbial activity and survival of microorganisms affected iodine sorption. Incubation of soil samples with varying levels of biomass, oxygen concentration, and soil water content showed the participation of soil microflora in iodine immobilization. Pure cultures of soil bacteria and fungi incorporated radioiodine (Bors et al., 1991). Radioiodine (^{125}I) adsorption by soil treated with nutrient showed elevated levels of microbial biomass with increased adsorption of radioiodine (Bors and Martens 1992). Anaerobiosis during the incubation period lowered adsorption. Migration of radioiodine in water saturated soil columns was influenced by the quantity of microorganisms present. Soils high in organic substance and soil biomass exhibited higher radioiodine sorption compared to clay minerals; and the iodine sorption process was predominantly irreversible (Bors et al., 1991).

In terrestrial environments iodine concentrations accumulated in soils average of 5 mg kg^{-1} world-wide which is much higher than those of their parent materials such as rocks and plants (0.05 to 0.5 mg kg^{-1}). Similarly, iodine concentrations in certain marine sediments are high (100 to 2000 mg kg^{-1}) compared with that in seawater (0.06 mg L^{-1}). Such high iodine accumulation in soils and sediments has been attributed to at least in part to microbial effects, although the mechanism of accumulation process is not fully understood. One possible explanation for this accumulation of iodine is that the iodide ion (I^-) is actively transported into the bacteria isolated from the marine sediment which accumulated iodide >5000-fold (Amachi et al., 2002; 2005). Iodide adsorption by the Gram-positive soil bacterium *Bacillus subtilis* showed that positively charged single sites on the cell wall were responsible for iodide sorption onto the surface of *B. subtilis* with a concentration of $3.54 \pm 3.80 \mu mol \text{ iodide } g^{-1} \text{ bacteria}$. Uptake and accumulation of iodide in washed cell suspensions of marine bacteria increased with the addition of glucose, while iodate was not accumulated by the bacteria (Amachi et al., 2002; 2005). Although a wide variety of terrestrial and marine bacteria has the potential for fixation of iodine in the environment there is very little information on the chemical speciation of the bioaccumulated iodine in bacteria and also the long term-stability of such species.

10. BIOCOLLOIDS

Microbes play an important role in the generation (formation) and destabilization of both intrinsic and pseudo colloids. Microbes bioaccumulate and biosorb radionuclides intracellularly or extracellularly. They themselves by virtue of their small size (1 nm to 1 μm) can form biocolloids and can affect the transport of radionuclides (Francis et al., 1998). Growing bacteria are several micrometers long with volumes of several cubic microns, while non-growing bacteria, under oligotrophic conditions, at a minimum may be as small as 0.2 - 0.3 μm with a volume not less than 0.05 μm^3 . In addition, biomass accumulated on surfaces (biofilms) can

become detached and generate biocolloids. Microorganisms are important sources of organic colloids, as are their metabolic by-products and exocellular polymers.

11. SUMMARY

Microorganisms play a major role in the transformations of radionuclides generated from nuclear fuel processing plants and regulate the mobility and stability of the radionuclides in the environment. The key microbial processes involved in the mobilization or immobilization of selected radionuclides of the interest are summarized in Table 1.

Table 1. Summary of Microbial Transformations of Radionuclides Released from Nuclear Fuel Processing Plants

Process	U	Np	Pu	Am	Tc	I	Cs	Sr
Oxidation ¹	++	ND	ND	NA	+	++	NA	NA
Reduction ²	++++	+?	+?	NA	+++	++	NA	NA
Dissolution ³	+++	?	+	?	+++	++	+	+
Precipitation	+++	+	++	?	+++	++	?	++
Biosorption	++++	+	++	+	?	++	++	++
Biomethylation	NA	NA	NA	NA	NA	+++	NA	NA
Biocolloid ⁴	++	+	+	+	?	?	?	?

NA- not applicable; ND- not determined.

¹Dissolution due to oxidation from lower to higher valence state.

²Reductive precipitation due to enzymatic reduction from higher to lower valence state.

³Dissolution due to oxidation from lower to higher valence state, changes in pH, production of organic acids and sequestering agents.

⁴Association of radionuclides with suspended bacteria, which can be transported as biocolloids.

Fundamental understanding of the mechanisms of microbial transformations of several chemical forms of the radionuclides in the presence of electron donors and acceptors under various environmental conditions such as aerobic and anaerobic (denitrifying, fermentative, sulfate-reducing, and methanogenic) will be useful in assessing the microbial impact on the long-term behavior of radionuclides released from nuclear fuel reprocessing plants and in developing appropriate management and remediation strategies for contaminated sites.

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